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DORSAL ROOT POTENTIALS AND CHANGES IN EXTRACELLULAR POTASSIUM IN THE SPINAL CORD OF THE FROG*

BY R. A. NICOLL

From the Departments of Pharmacology and Physiology, University of California, San Francisco, California 94143, U.S.A.

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SUMMARY

1. In the present study changes in extracellular potassium, ($[K]_e$), were recorded in the isolated spinal cord of the frog with glial cell recordings and K-selective micro-electrodes to test the hypothesis that elevations in $[K]_e$ during neuronal activity produce the dorsal root potential (d.r.p.).

2. Sucrose gap recording from the dorsal root (d.r.) was used to record responses to root stimulation and to exogenously applied K^+.

3. Stimulation of the ventral root, which elicits a d.r.p. in the frog spinal cord, was not associated with any change in $[K]_e$, suggesting that d.r.p.s produced by ventral root stimulation are not due to changes in $[K]_e$.

4. The largest change in $[K]_e$ observed following single stimuli to the dorsal root was 0·4 mM. Such a change in $[K]_e$, if evenly distributed, would depolarize the dorsal root by about 1 mV and yet the simultaneously recorded d.r.p. evoked by stimulating an adjacent dorsal root (d.r.-d.r.p.) was over 10 mV.

5. The time-to-peak of the glidal cell responses was 10 times that of the d.r.-d.r.p. Low frequency (1–10 Hz) d.r. stimulation caused a decremental summation of glial cell responses, while there was no summation in the d.r.-d.r.p. These results suggest that the d.r.p. produced by single d.r. stimulation is generated in large part by a mechanism other than a change in $[K]_e$.

6. During high frequency d.r. stimulation, which evoked 6–8 mM increases in $[K]_e$, the adjacent d.r. was depolarized to a greater extent than that produced by single stimuli. The magnitude of this depolarization was similar to that produced by applying a $[K]_e$ equivalent to that observed in the spinal cord during high frequency stimulation. Thus, a substantial component of the sustained d.r. depolarization during high frequency d.r. stimulation may result from changes in $[K]_e$.

7. In the presence of magnesium, high frequency d.r. stimulation evoked a picrotoxin resistant depolarization of an adjacent d.r. whose magnitude correlated well with the changes in $[K]_e$ recorded in the spinal cord.

8. In the presence of picrotoxin a slow, long duration depolarization of the d.r. occurred following single stimuli to the adjacent d.r. and the appearance and time course of this response correlated well with the time course of changes in $[K]_e$.

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9. Addition of K^+ to the Ringer solution in concentrations up to 12 mM had a facilitatory action on reflex activity in the frog spinal cord.

10. The present results suggest that although changes in $[K]_e$ play a relatively minor role in generating d.r.p.s elicited by single d.r. stimulation, the sustained dorsal root depolarization evoked either by high frequency stimulation or by single stimuli in the presence of picrotoxin may be due to a considerable extent to $[K]_e$.

INTRODUCTION

As a consequence of activity in spinal primary afferent fibres, the intramedullary portion of afferents in the vicinity of the activated fibres are transiently depolarized. This depolarization, which has been proposed to exert a presynaptic inhibition of primary afferents, can be recorded from the dorsal root as an electrotonic potential and is termed the dorsal root potential (d.r.p.) (Eccles, 1964; Schmidt, 1971).

Currently there are two major hypotheses to explain the generation of d.r.p.s. In their original description of d.r.p.s Barron & Matthews (1938) suggested that these potentials might arise from a transient shift in the ionic environment surrounding the primary afferents. This hypothesis has recently been revived by the introduction of potassium specific micro-electrodes to neurophysiological investigations (Krnjevic & Morris, 1972; Bruggencate, Lux & Liebl, 1974; Kriz, Sykova, Ujec & Vyklicky, 1974; Kriz, Sykova & Vyklicky, 1975; Lothman & Somjen, 1975; Sykova, Shirayev, Kriz & Vyklicky, 1976). Specifically it is proposed that increases in extracellular potassium during neuronal activity depolarize afferent fibres.

The alternative proposal is that primary afferents activate interneurones which make specific axo-axonic synapses on the terminals of primary afferents (Eccles, 1964). The axo-axonic synapse releases a transmitter that depolarizes the afferent terminal. The demonstration of axo-axonic synapses by electron microscopy (Conradi, 1969; Rethelyi, 1970) and evidence from pharmacological (Eccles, 1964; Davidson & Southwick, 1971; Davidoff, 1972; Barker & Nicoll, 1973; Nishi, Minota & Karczmar, 1974), chemical (Miyata & Otsuka, 1972), and histochemical studies (McLaughlin, Barber, Saito, Roberts & Wu, 1975) provide support for this specific synaptic mechanism, and suggest that γ-aminobutyric acid (GABA) is the depolarizing transmitter released from the axo-axonic synapse (Burke & Rudomin, 1977; Levy, 1977; Nicoll & Alger, 1979).

The present investigation examines the possibility that increases in extracellular K contribute to the d.r.p. in the frog spinal cord. Glial cell recording and potassium specific micro-electrodes have been used to monitor changes in extracellular K^+ ($[K]_e$). In addition the effect of K^+ on synaptic transmission has been examined. A brief account of some of these results has appeared (Nicoll, 1976).

METHODS

The isolated hemisected frog spinal cord was used in these experiments. A sucrose gap chamber was used to record from the dorsal root (Barker *et al.* 1975*a*). The dorsal root was placed tightly in the slot leading to the sucrose compartment so that only the intramedullary portion of the primary afferents was perfused with the Ringer solution.

The Ringer solution consisted of 115 mM-NaCl, 2 mM-$CaCl_2$, 2 mM-KCl, and 10 mM-Tris (hydroxymethyl) aminomethane buffer adjusted to pH 7·3. The Ringer solution was bubbled with 100% O_2. The temperature was maintained at 18 °C unless stated otherwise. K^+-rich solutions were made by substituting K^+ for Na^+. It was not possible to use a Cl-free Ringer for experiments involving changes in $[K]_e$, which would prevent any water flux resulting from movement of KCl as the $[K]_e$ was changed (Boyle & Conway, 1941), since such a Ringer grossly altered the physiology of the frog spinal cord (Katz & Miledi, 1963; Barker & Nicoll, 1973). However, the K^+ changes in the present experiments could be made repeatedly for many hours without any deleterious effect on the spinal cord. Solution changes were made by activating a solenoid valve (General Valve).

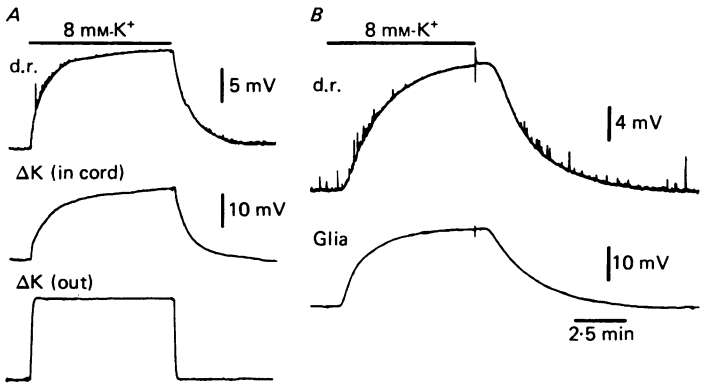

Fig. 1. Effect of $[K]_e$ on primary afferents and glial cells. *A*, the top record shows sucrose gap record from the dorsal root (d.r.); the middle record was obtained from a K-selective micro-electrode positioned in the midst of the primary afferents; the bottom record was obtained after withdrawing the electrode into the superfusate. *B*, the top record shows sucrose gap record from the d.r. of the depolarization induced by 8 mM-K. The bottom trace is a simultaneous record from a glial cell.

For intracellular recording glass micropipette electrodes filled with 3 M-KCl (10–30 MΩ impedance) were used and the signal was recorded with a WPI model M701 amplifier. The usual electrical criteria were used to distinguish neuroglial cells from neurones. Glial cells did not generate action potentials or synaptic potentials either to direct intracellular or synaptic stimulation of the dorsal root. Although the membrane potential of glial cells was unusually stable, often for periods of more than an hour, their membrane potential rarely exceeded 80 mV. It is possible that hemisection of the cord damaged processes of the glial cell leading to the low membrane potentials. Only those cells with stable potentials over 60 mV were used.

Pipettes, containing a K-selective liquid ion exchanger, were also used to record K^+ activity of the extracellular fluid and were purchased from Microelectrodes, Inc. The signal from these electrodes was led through a capacity-compensated high input impedance (10^{13} Ω) preamplifier. A second pipette, containing 3 M-NaCl with an impedance of 10 MΩ was positioned within 20 μm of the K-specific electrode using a Narashige MT-5 micromanipulator. With this manipulator one can position under a microscope the tips of two electrodes with precision. The difference in the potential between the two electrodes was used to record the K^+ activity within the spinal cord. The electrodes were calibrated with known concentrations of K^+ before and after measurements were obtained in the spinal cord.

To test the effects of K^+ on synaptic transmission, care had to be taken that electrotonic spread of the K^+ induced depolarization to the site of d.r. stimulation did not increase the number of fibres activated. 4–6 cm of sciatic nerve were dissected so that the stimulating electrode was far removed from the spinal cord. In those experiments where transmission was partially blocked with Mg^{2+}, supramaximal stimuli were applied to the primary afferents so that any increase in excitability at the stimulating site would not result in the activation of more primary afferents.

RESULTS

Sensitivity of d.r. to changes in $[K]_e$. Fig. 1*A* and *B* show the response of the primary afferents to a Ringer containing 8 mM-K^+. A depolarization of 13 mV was recorded when the response had equilibrated. The graph in Fig. 2 plots the results of responses obtained from a number of preparations to various concentrations of K^+. The question arises as to whether the concentration of K^+ in the vicinity of the

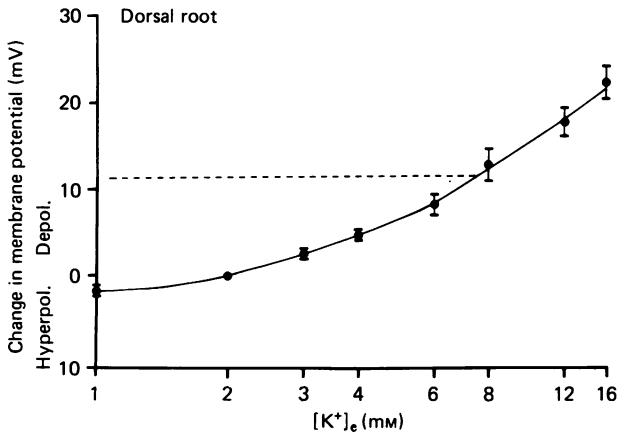

Fig. 2. Effect of changing $[K]_e$ on the membrane potential of primary afferents. 2 mM is the concentration present in the control Ringer solution. The ordinate is the change in potential induced by the test solution. Each point represents from four to fifteen determinations in separate preparations and the bars represent the standard deviation.

primary afferents is the same as that in the perfusate. To address this issue K-specific electrodes were positioned in the dorsal horn in the region which gave the maximum primary afferent field potential. The change in K^+ activity recorded at this site (Fig. 1*A*, middle record) was considerably slower than that recorded when the electrode was withdrawn from the tissue (Fig. 1*A*, bottom record), and had a time course similar to the response of the dorsal root, supporting the notion that the location of the tip of the K-specific electrode was in the same region as the primary afferents. The maximum value obtained when the response had equilibrated was close to that found in the Ringer solution. The average difference obtained from a number of determinations was 6%.

The average size of the dorsal root potential derived from stimulating the adjacent dorsal root (d.r.-d.r.p.) was 11·4 mV ($n = 35$ S.D. 1·2), while that obtained from stimulating the ventral roots (v.r.-d.r.p.) was 3·2 mV ($n = 13$). From the graph in Fig. 2 it can be calculated that a change of approximately 5 mM would be required to produce a d.r.-d.r.p. of the size observed, while a change of 1·5 mM would be needed for the v.r.-d.r.p. This reasoning assumes that all the afferents in the dorsal root are exposed to the same concentration of K^+. An uneven distribution of K^+, which is more likely to occur following dorsal root stimulation, would require local changes in excess of 5 mM.

The values obtained from sucrose gap recording are inevitably somewhat less than the true transmembrane potential change which would be recorded with an intracellular micro-electrode. Application of isotonic KCl depolarized the primary afferent approximately 60 mV. Assuming that the true resting potential is about −70 mV, this result suggests that the values obtained from the present recording system are underestimated by about 15%. The values reported above have not been corrected, because the purpose of the present study was to compare the effect of K to d.r.p.s recorded in an identical fashion.

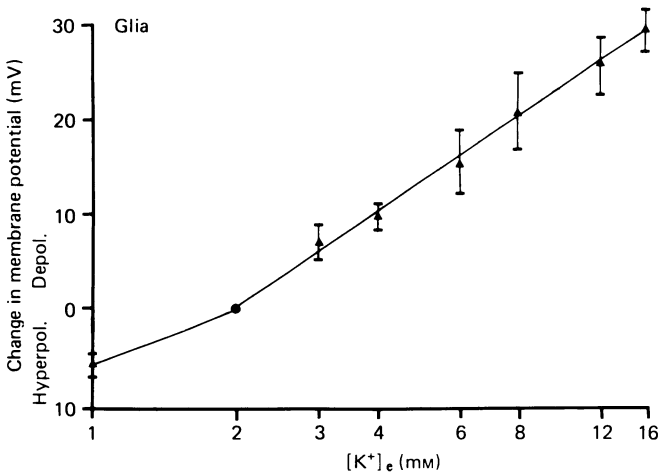

Fig. 3. Effect of changing $[K]_e$ on the membrane potential of glial cells. 2 mM is the concentration present in the control Ringer solution. The ordinate is the change in membrane potential induced by the test solution. Each point represents from three to twelve determinations and the bars represent the standard deviation.

Responses of glial cells to changes in $[K]_e$ *and root stimulation.* The use of glial cell recordings to monitor changes in $[K]_e$ should have an advantage over the use of K-specific micro-electrodes, in that the existence of a dead space around the tip of the K-specific micro-electrode makes interpretation of the time course and magnitude of the changes difficult.

Glial cells were penetrated in the dorsal horn and intermediate regions of the spinal cord in areas which elicited large focal responses to dorsal root stimulation and, therefore, are likely to be areas with maximal changes in $[K]_e$. Thus, the recordings were usually made in the spinal cord adjacent to the dorsal root that was stimulated, rather than adjacent to the dorsal root in the sucrose gap. Before a cell was tested for its sensitivity to K^+ a stable membrane potential for at least 5 min was required. The Ringer with altered K^+ concentration was then applied until a steady membrane potential was reached at which time the preparation was returned to the standard Ringer solution (Fig. 1*B*, bottom record). Usually repolarization was slower than the depolarization, as has been observed for glial cells in other systems (Kuffler, Nicholls & Orkand, 1966; Ransom & Goldring, 1973*a*). Some cells were not held long enough for a complete recovery. If the response had reached a stable plateau level in the test Ringer and began to repolarize upon reinstituting the control Ringer, the values were included in the graph in Fig. 3. The line in Fig. 3

has a slope of 34 mV for a tenfold change in $[K]_e$. This is considerably less than the expected slope of 58 mV for a membrane potential that is solely determined by the K^+ gradient. The most likely explanation for this discrepancy is the fact that the resting potential of the cells used in the present experiments was less than that recorded in most previous studies (see Methods).

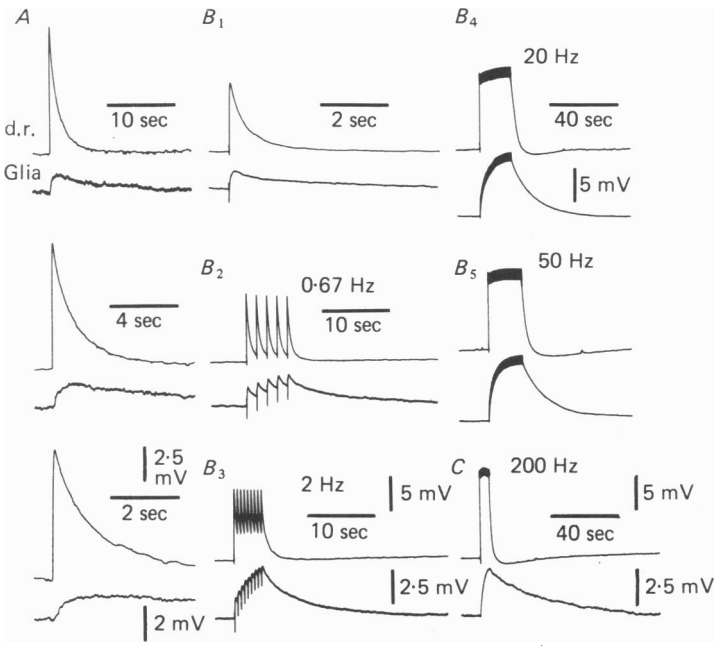

Fig. 4. Comparison of dorsal root potential and glial depolarization induced by dorsal root stimulation. In each pair of records the top record was obtained from a dorsal root and the bottom record from a glial cell. *A* shows responses at different chart speeds. The membrane potential of the glial cell was 73 mV. The gain calibrations in the bottom records also apply to the other traces. Preparation was at 12 °C. *B* is from another preparation maintained at 18 °C showing that during repetitive stimulation the glial potential sums decrementally while the dorsal root potential falls off slightly at lower frequencies. The glial depolarization reached maximum values at 20 Hz, there being no further increase at 50 Hz. The gain calibration in the bottom pair of records also applies to the upper records. *C* shows responses from another preparation. The glial membrane potential was 68 mV. The temperature was 14 °C.

Although the magnitude of the change in $[K]_e$ recorded in the spinal cord with K^+-sensitive electrodes agrees reasonably well with the changes in the bath, it is conceivable that the sensitivity of the glial cells has been underestimated because the change of K^+ in the narrow extracellular space is less than that measured with the K^+-sensitive electrode. This would lead to a substantial over-estimation of the $\Delta[K]_e$ calculated from changes in glial cell membrane potential. However, the magnitude of the $\Delta[K]_e$ generated by neuronal activity and calculated from the glial recordings agrees quite well with the values obtained with K^+-sensitive electrodes in this and other studies.

Changes in $[K]_e$ during spinal root stimulation. Virtually all glial cells responded with a slow depolarization following single stimuli to the dorsal root (Figs. 4 and 5).

The average depolarization was 1·2 mV ($n = 23 \pm$ S.D. 0·5). The largest response observed was 2·5 mV. This is equivalent to a change in K^+ of approximately 0·4 mM which would, if uniformly distributed, be expected to depolarize the dorsal root by 1 mV. The average time to peak of the glial response was 0·7 sec compared to 20–35 msec for the d.r.-d.r.p. The average time for the glial response to decline to half was 4·6 sec compared to 0·55 sec for the d.r.-d.r.p. The most rapid response seen in the present study reached a peak in 250 msec and declined to half in 1·2 sec.

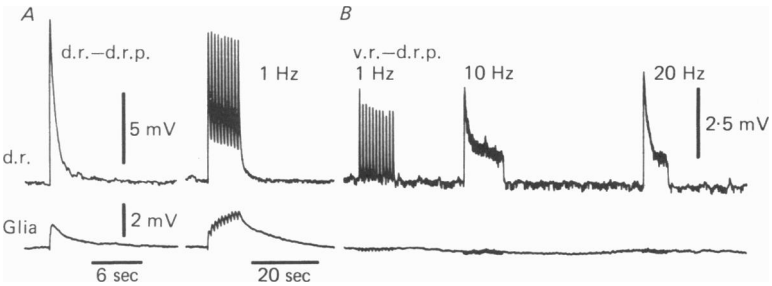

Fig. 5. Comparison of dorsal and ventral root stimulation on glial potential. *A* shows responses to dorsal root stimulation and *B* to ventral root stimulation. The gain for the glial record was the same throughout while for the dorsal root record the gain was doubled during ventral root stimulation. Glial cell membrane potential was 77 mV.

The glial cell response will represent an average value for the $\Delta[K]_e$ occurring in the region where the cell was penetrated. The recordings were generally made in the segment of the stimulated dorsal root. Presumably not all the afferents in the adjacent dorsal root, from which the d.r.p. was recorded, will be exposed to this $\Delta[K]_e$. Thus, the calculated value of 1 mV depolarization, which is based on a uniform exposure of the primary afferents to K^+, may be an over-estimation.

Repetitive dorsal root stimulation resulted in a decremental summation of the glial responses (Figs. 4*B* and 5*A*), and the response recorded from the K-specific micro-electrode, as has been reported in a number of other systems (Orkand, Nicholls & Kuffler, 1966; Ransom & Goldring, 1973*b*). No such decremental summation occurred for d.r.-d.r.p.s and at low frequency stimulation the peak of the d.r.-d.r.p.s often decreased slightly. With higher frequency stimulation the d.r.-d.r.p.s partially fused resulting in a sustained depolarization of the primary afferents, which with frequencies above 10–20 Hz usually resulted in a peak depolarization that was larger than that produced by a single d.r.-d.r.p. (Fig. 6*A*). Glial membrane depolarizations of up to 20 mV, which is equivalent to a $[K]_e$ of 8 mM, were seen with tetanic stimulation. Similar measurements of $[K]_e$ were obtained with K-specific micro-electrodes. The change in $[K]_e$ evoked by tetanic stimulation required seconds to plateau and gradually subsided to control values with a half-time of approximately 10–20 sec following termination of the stimuli. A hyperpolarizing phase was not seen during recovery. Following termination of tetanic stimulation, the dorsal roots repolarized rapidly with a half time of about 1–2 sec. Recovery from high frequency stimulation was accompanied by a transient hyperpolarization of the dorsal root (Fig. 4*B* and *C*).

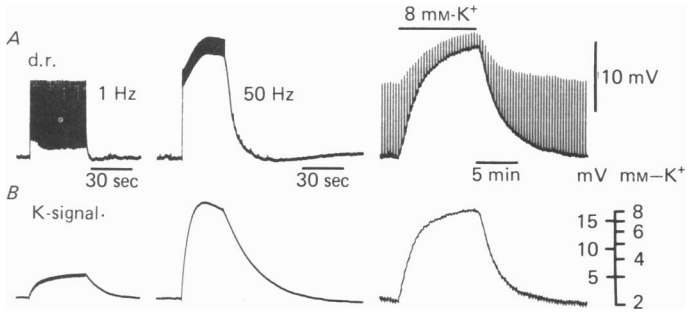

Fig. 6. Comparison of repetitive dorsal root stimulation and changes in $[K]_e$ on dorsal root potentials. *A* shows sucrose gap recording from the dorsal root (d.r.) and *B* shows the response record simultaneously from a K-selective micro-electrode. The left hand column shows the responses to 1 Hz dorsal root stimulation and the middle column shows the responses to 50 Hz dorsal root stimulation. In the right hand column is the response to an 8 mM-K^+ Ringer solution.

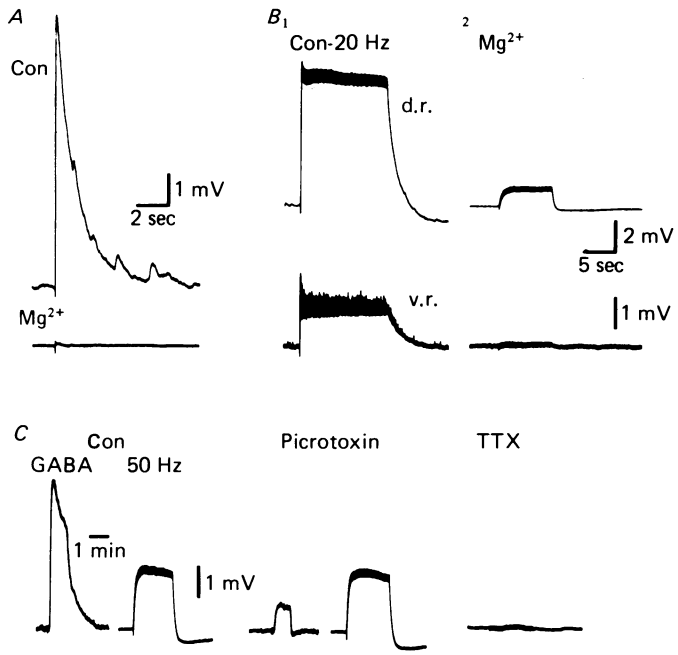

Fig. 7. The effect of Mg^{2+} ions on responses induced by dorsal root stimulation. *A* shows a d.r.-d.r.p. before (Con) and after addition of 10 mM-Mg^{2+} ions. B_1: control responses from the dorsal (d.r.) and ventral (v.r.) root to 20 Hz stimulation of an adjacent dorsal root. B_2: effect of 10 mM-Mg^{2+} ions on these responses. *C* shows that in the presence of 10 mM-Mg^{2+} ions picrotoxin antagonizes the response to GABA on the dorsal root but has no effect on the response to 50 Hz stimulation of the adjacent dorsal root, while tetrodoxin (TTX) in a concentration of 5×10^{-6} M blocks the response to dorsal root stimulation.

In Fig. 6*A* a comparison has been made between the effect of tetanic stimulation, which causes a $\Delta[K]_e$ of 6 mM, and the effect of an equivalent concentration of exogenously applied K^+. It can be seen that the peak of the d.r.-d.r.p. during the K^+ infusion reaches the same absolute level of depolarization as during tetanic stimula-

tion and that the size of the individual d.r.-d.r.p.s during the tetanus is similar to those recorded at the peak of the K^+-induced depolarization.

Stimulation of the ventral roots, either with single or high frequency stimulation, failed to alter the membrane potential of glial cells (Fig. 5*B*) and yet these same stimuli invariably resulted in a depolarization of the dorsal root (v.r.-d.r.p.).

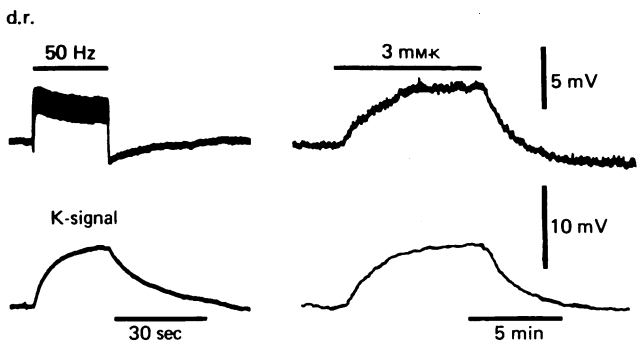

Fig. 8. Comparison of the depolarization of the dorsal root by dorsal root stimulation and by K^+ in the presence of Mg^{2+} ions. The records on top were recorded with sucrose gap from the dorsal root, while those on the bottom were recorded with a K-specific micro-electrode. The magnitude of the d.r. depolarization evoked by 50 Hz stimulation of an adjacent d.r. is similar to that produced by applying a $[K]_e$ equivalent to that observed in the spinal cord during the 50 Hz stimulation.

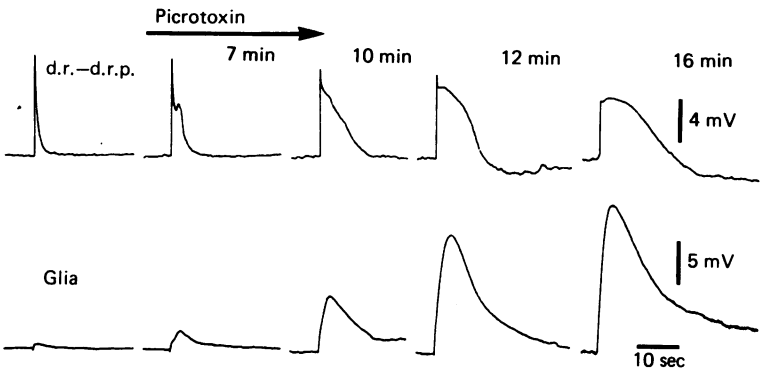

Fig. 9. Effect of picrotoxin on glial and dorsal root potentials. The responses on the left are controls. Picrotoxin (5×10^{-4} M) was added to the Ringer and the records to the right were obtained as the picrotoxin exerted its effect. The glial membrane potential was 62 mV.

Origin of the change in $[K]_e$ *during d.r. stimulation.* Changes in $[K]_e$ could result from action potentials in primary afferents, interneurones and motoneurones. The failure to alter K^+ during ventral root stimulation suggests that the soma and axons of motoneurones do not contribute to $\Delta[K]_e$ seen during d.r. stimulation. It is possible that the dendrites, which are poorly invaded with antidromic stimulation, do contribute to the orthodromic response. To examine the possible contribution of primary afferents to $\Delta[K]_e$, synaptic transmission was blocked by adding Mg^{2+} (10 mM) to the Ringer. This resulted in the virtual abolition of the d.r.-d.r.p. to single stimuli (Fig. 7*A*). However, with d.r. repetitive stimulation the adjacent root, but

not the ventral, was depolarized by approximately 1 mV (Fig. 7*B*). This depolarization was not due to residual synaptic release of GABA, since picrotoxin had no effect on this response (Fig. 7*C*). The response was dependent on action potentials in primary afferents since removal of Na from the Ringer (Tris substitution) or addition of tetrodotoxin entirely blocked the response (Fig. 7*C*).

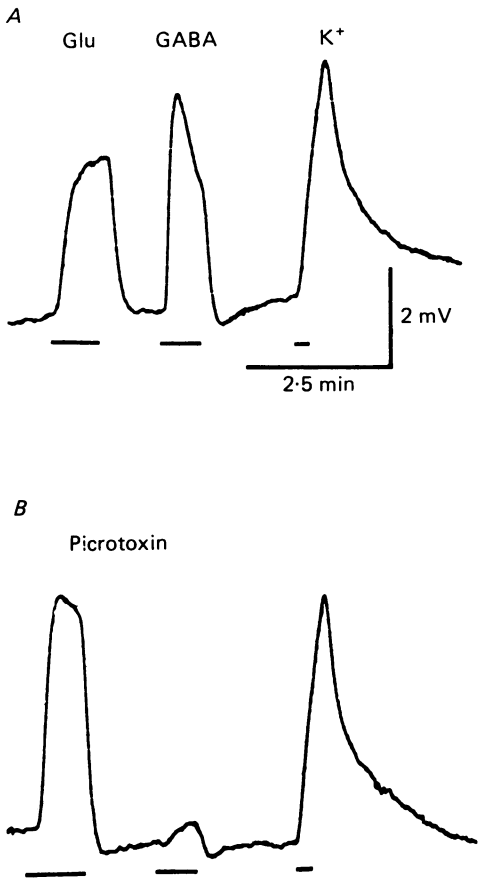

Fig. 10. Effect of picrotoxin on the K^+ depolarization of the dorsal root. The recording in *A* shows the control responses to glutamate (GLU), GABA, and K^+. *B* was obtained 10 min after introducing 5×10^{-4} M-picrotoxin into the Ringer. Amino acids were applied in a concentration of 2×10^{-4} M and K^+ in a concentration of 8 mM.

As reported by Sykova & Vyklicky (1977) K-specific electrodes detected a change in $[K]_e$ of approximately 1 mM which could fully account for the magnitude of the d.r. depolarization seen in the presence of Mg^{2+} (Fig. 8). However, the time-to-peak of the depolarization was always less than that of the change in $[K]_e$. In addition, the dorsal root transiently hyperpolarized after terminating the stimulus, while the K signal slowly returned to control valves.

Effect of picrotoxin on $[K]_e$ *during d.r. stimulation.* As reported previously (Barker *et al.* 1975*b*) picrotoxin antagonizes the d.r.-d.r.p., but a much slower, picrotoxin resistant depolarization appears in the presence of picrotoxin. Simultaneous recording

from the d.r. and glial cells (Fig. 9) indicates that the appearance of this late depolarization is precisely timed with the appearance of a large glial cell depolarization which has a similar time course. The magnitude of this response is equivalent to a change in $[K]_e$ of 6·5 mM which would be expected to depolarize the d.r. by 10 mV. Since picrotoxin does not antagonize the depolarizing action of K^+ on the dorsal root (Fig. 10) this marked increase in $[K]_e$ seen in the presence of picrotoxin could produce the slow picrotoxin resistant depolarization.

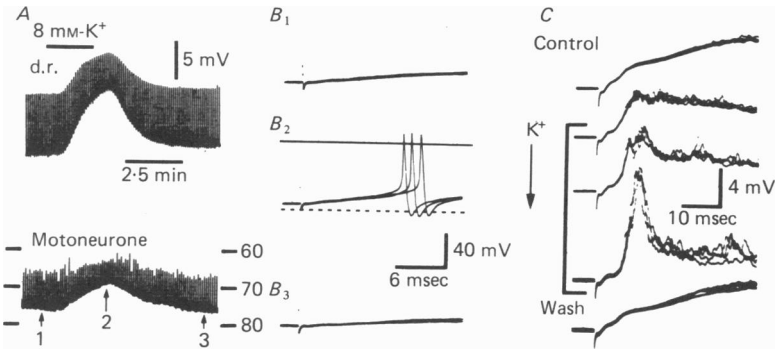

Fig. 11. Effect of K^+ on excitation of motoneurones by dorsal root stimulation. In *A* the top chart record is from the dorsal root and the bottom intracellular record is from a motoneurone. An adjacent dorsal root was stimulated every 5·5 sec with a stimulus intensity which was just below threshold for activating the motoneurone. *B* shows film records obtained before (1), during (2) and after (3) exposing the cord to 8 mM-K. The responses in *C* are ventral root potentials to maximum dorsal root stimulation (d.r.-v.r.p.) recorded with sucrose gap. The top record is the control; the middle during 8 mM-K; the bottom after recovery.

Effect of K^+ on synaptic transmission. To examine the possible consequences of $\Delta[K]_e$ on synaptic transmission, K^+ enriched Ringer was applied to the cord. In concentrations up to 8 mM, K^+ caused an increased efficacy of transmission. Thus the ventral root reflex elicited by dorsal root stimulation (Fig. 11*C*) and the dorsal root reflex elicited by dorsal root stimulation were markedly enhanced by K^+. In addition, K^+ could partially reverse the block in synaptic transmission produced by 5 mM-Mg^{2+}. Intracellular recording from motoneurones also demonstrated that subthreshold e.p.s.p.s (excitatory post-sympatic potentials) derived from primary afferent stimulation surpassed threshold in the presence of K^+, despite the fact that the primary afferent terminals were depolarized (Fig. 11*A* and *B*).

DISCUSSION

The present experiments using glial cells and K-selective micro-electrodes to measure changes in extracellular potassium were designed to test the hypothesis that elevations in extracellular potassium during and following neuronal activity can account in part or entirely for the generation of d.r.p.s. The d.r.p. represents an average response of the depolarization that occurs in each of the fibres in the dorsal root. Since it is likely that glial cell processes sample K^+ activity over a large area, especially if glial cells are electrically coupled (cf. Kuffler *et al.* 1966), glial cell

responses would also provide an average value for $\Delta[K]_e$. The distance between the site of maximum $\Delta[K]_e$, which would be in the spinal segment of the stimulated d.r., and the glial recording, also obtained from the stimulated segment, would certainly be no greater than the distance between site of the maximum $\Delta[K]_e$ and the recording site on the adjacent d.r. Thus the glial recording site would be as favourable, or indeed probably more favourable, for detecting the $\Delta[K]_e$ than would be the majority of afferents in the adjacent d.r. Finally glial cells are as closely opposed to primary afferents as are primary afferents to each other and therefore should provide a good index of the $\Delta[K]_e$ occurring in the immediate vicinity of afferent membranes. Thus a comparison of the responses recorded from glial cells and the dorsal root should provide a reasonable test of the hypothesis that d.r.p.s result from $\Delta[K]_e$ around primary afferent fibres (cf. Lothman & Somjen, 1975).

The present results demonstrate that there is no $\Delta[K]_e$ associated with the generation of the v.r.-d.r.p., thus making it extremely unlikely that such a process contributes to the generation of this response. The basis for there being no $\Delta[K]_e$ following v.r. stimulation, which is in agreement with other findings (Kriz *et al.* 1974; Sykova *et al.* 1976), may be due to blockade of soma-dendritic invasion during repetitive stimulation. The dense arborization of the primary afferent fibres (Szekely, 1976) would provide a larger surface area for potassium release than would the motor axons.

In response to single d.r. stimulation there is a transient glial cell depolarization in the vicinity of primary afferents. The largest response observed was 2·5 mV which is similar to that observed in other systems (cf. Orkand *et al.* 1966; Ransom & Goldring, 1973*b*) and was equivalent to an average, apparent change in $[K]_e$ of 0·4 mM. This should depolarize the d.r. approximately 1 mV and yet the d.r.-d.r.p. averaged 11·4 mV. This suggests that following single stimuli not more than about 10% of the d.r.-d.r.p. at its peak could be attributed to a change in $[K]_e$. The slow time course for the $[K]_e$ suggests that K^+ may contribute more to the falling phase of the d.r.-d.r.p. (cf. Krnjevic & Morris, 1975).

Low frequency (1–10 Hz) dorsal root stimulation invariably caused a decremental summation of the changes in $[K]_e$ while the peaks of the d.r.-d.r.p.s either remained constant or actually decreased. This clear discrepancy in the behavior of these two events supports the conclusion that they are not generated primarily by a common process. The evidence reviewed in the Introduction would favor a specific axo-axonic mechanism involved in dorsal root depolarization following single dorsal root stimulation.

High frequency dorsal root stimulation resulted in large increases in $[K]_e$. When the increases were great enough to be expected to depolarize the dorsal root to a larger extent than that seen with a single d.r.-d.r.p., the summated d.r.-d.r.p.s during the tetanus reached a more depolarized level than that seen at the peak of a single d.r.-d.r.p. The duplication of these effects by applying a $[K]_e$ equivalent to that observed in the spinal cord suggests that during high frequency stimulation up to 80–90% of the sustained depolarization of the d.r. may be due to the accumulation of $[K]_e$. The rapid recovery and hyperpolarization of the primary afferents following tetanic stimulation, which is not observed with the $[K]_e$, could be explained by the activation of an electrogenic Na pump by dorsal root reflex activity which occurs throughout the stimulus period.

Two observations support the hypothesis that during high frequency neuronal activity induced by stimulating one dorsal root, the primary afferents entering through an adjacent dorsal root do see the resulting $\Delta[K]_e$. First, the addition of the GABA antagonist picrotoxin, which reduces the early, presumably GABA mediated component of the d.r.-d.r.p., results in the appearance of a much slower depolarization (Barker *et al.* 1975*b*; Lothman & Somjen, 1976). Simultaneous measurements of $[K]_e$ indicate that the time of appearance and the magnitude of this picrotoxin resistant depolarization correlate well with the observed $\Delta[K]_e$. The large change in $[K]_e$ seen in the presence of picrotoxin is presumably due to the marked increase in neuronal excitability resulting in intense high frequency neuronal discharges. Secondly, tetanic stimuli to the dorsal root in the presence of Mg^{2+} results in a small picrotoxin resistant depolarization of the adjacent dorsal root. The $\Delta[K]_e$ measured under such conditions is sufficient to cause the observed depolarization (cf. Vyklicky, Sykova & Mellerova, 1976; Sykova & Vyklicky, 1977).

The accumulation of $[K]_e$ during neuronal activity raises the important question concerning the effects that this accumulation might have on the efficacy of synaptic transmission. The net effect will depend upon the relative pre- and post-synaptic actions. While transmitter release appears to be reduced at the squid giant synapse (Weight & Erulkar, 1976; Erulkar & Weight, 1977), at the neuromuscular junction (Takeuchi & Takeuchi, 1961; Parsons *et al.* 1965) and at frog sympathetic ganglia (R. A. Nicoll, unpublished observations) quantal content is actually increased, and elevated $[K]_e$ can increase spontaneous transmitter release independent of membrane potential (Cooke & Quastel, 1973). Furthermore, K^+ greatly augments the efficacy of transmission at partially curarized neuromuscular junctions (Wilson & Wright, 1937; Brown & von Euler, 1938; Walker & Laporte, 1947). In the lateral geniculate nucleus depolarization of optic tract terminals, which may result from K^+ accumulation, is not associated with any change in the size of the e.p.s.p.s generated by these fibres (Singer & Lux, 1973). The over-all effect of K^+ in the present study of the frog spinal cord was to greatly facilitate reflex activity. The precise site at which this effect occurs remains to be elucidated but is presumably due in large part to the depolarization of post-synaptic membranes.

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